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(71) Applicant *(for all designated States except US)*: **BLANCHETTE ROCKEFELLER NEUROSCIENCES INSTITUTE** [US/US]; 9601 Medical Center Drive, Rockville, MD 20850 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **SUN, Miao-Kun** [US/US]; 16012 Howard Landing Drive, Bethesda, MD 20878 (US). **ALKON, Daniel, L.** [US/US]; 6701 Bonaventure Court, Bethesda, MD 20817 (US).

(74) Agent: **GOLLIN, Michael, A.**; Venable, Baetjer, Howard & Civiletti, LLP, 1201 New York Avenue, NW, Suite 1000, P.O. Box 34385, Washington, DC 20043-9998 (US).

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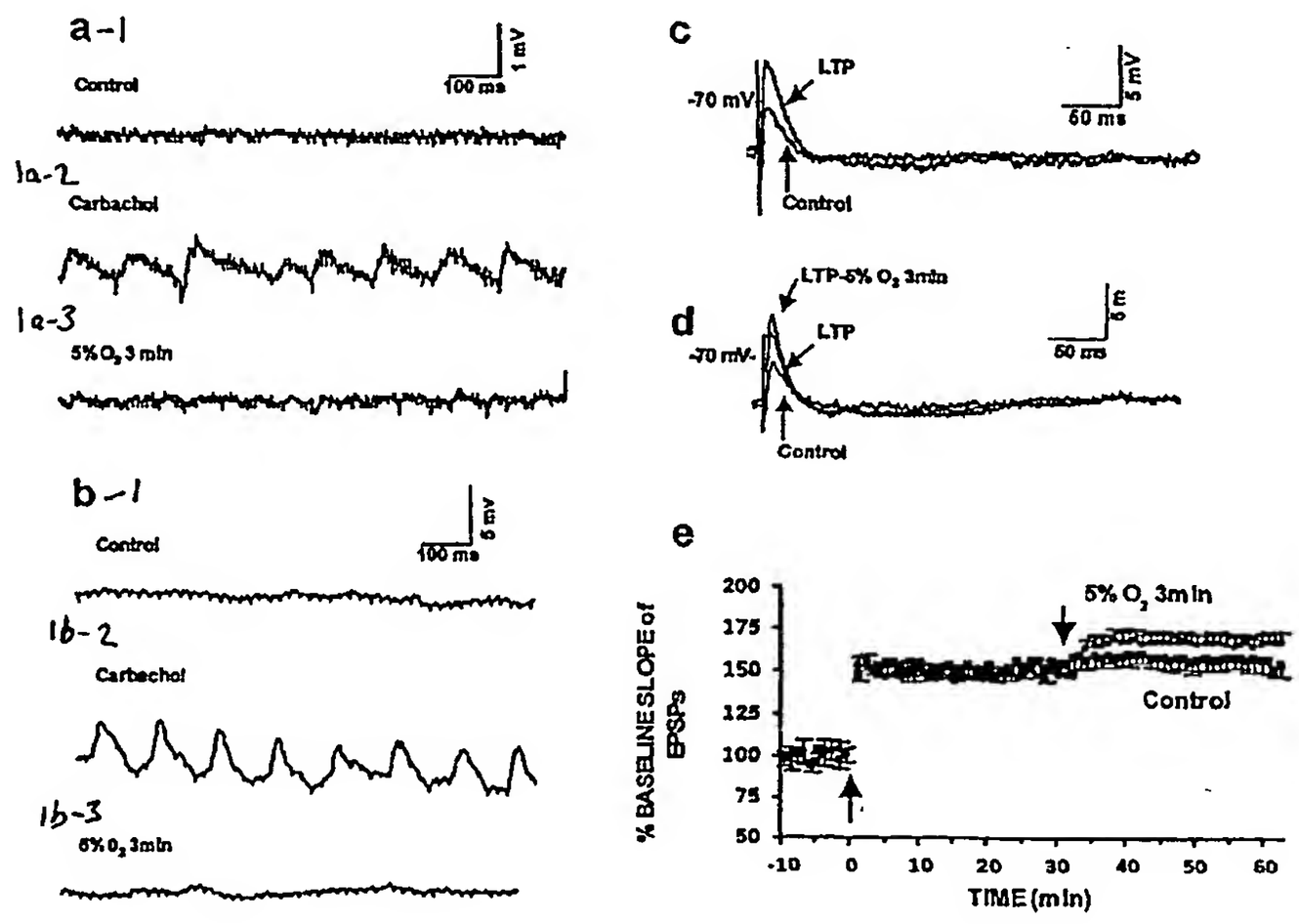

(57) Abstract: The invention provides therapeutic methods and compositions comprising adenosine A1 antagonists for treating memory loss produced by reversible transient hypoxia and associated synaptic dysfunction.

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ADENOSINE A1 RECEPTOR ANTAGONISTS FOR TREATING HYPOXIA-INDUCED LEARNING AND MEMORY IMPAIRMENT

This application claims the benefit of provisional application USSN 60/289,137, filed May 8, 2001, incorporated herein by reference.

FIELD OF THE INVENTION

The invention relates to a method for treating or preventing memory loss produced by reversible transient hypoxia-induced and associated synaptic dysfunction comprising administering an adenosine A1 receptor antagonist.

BACKGROUND

Hypoxia and ischemic stroke remain one of the most devastating threats to humans. Memory impairment is common after cerebral hypoxia/ischemia, bypass surgery, or heart attack[1]. Although all mammalian cells can sense and will respond to hypoxia[2,3], hippocampal CA1 pyramidal cells are among, if not the, most sensitive. Hypoxic/ischemic consequences consist mainly of three forms: functional disruptions, cellular injury and delayed cell loss through apoptosis[4] or necrosis, depending on the severity of the insult. Each form has distinct pathophysiological characterization and requires different therapeutics.

It is known that a selective adenosine A1 receptor antagonist, DPCPX, mitigates hypoxia-induced accumulation of adenosine during hypoxia. Pearson T. et al., Eur. J. Neurosci. 2000, 12(8):3064-6. Adenosine suppresses synaptic responses in rat hippocampus during hypoxia, and that suppression was reversed by use of an A1 antagonist. Arlinghaus et al., Brain Res. 1996, 724(2):265-8. Adenosine-mediation of anoxia induced synaptic glutamate release in CA1 pyramidal neurons was not affected by DPCPCX. Katchman et al., Hippocampus 1996, 6(3):213-24. and U.S. Patent 6,166,181. Another antagonist blocked hypoxia-induced depression of synaptic transmission in CA1 neurons. Doolette et al., Brain Res. 1995, 677(1):127-37. These references do not demonstrate or teach any effect of hypoxia on hippocampal theta rhythm, attention, learning, or memory, or an effect of blockade of CA1 adenosine A1 receptors in preventing hypoxia-induced memory loss.

Spatial learning and memory depend on information processing by the hippocampal networks, whose function is extremely sensitive to mild hypoxia and transient ischemia. However, despite intensive research aimed at the development of effective therapeutic interventions, promising therapy is still lacking. The present invention demonstrates that

reversible transient hypoxia reduced cholinergic θ activity and associated synaptic "arrest' in hippocampal CA1, and that these responses were preventable by adenosine A_1 receptor antagonism. Brief hypoxic episodes markedly impaired the ability of rats in a Morris water-maze spatial learning and memory. The impairment was prevented by adenosine A_1 receptor antagonism. This protection of synaptic efficacy represents an effective therapeutic strategy to eliminate functional interruption due to brief hypoxic episodes. Moreover, the present invention provides a molecule with high log P and that readily enters the central nervous system or one that may be transferred or "locked" into the brain through the use of a pro-drug technique.

SUMMARY OF THE INVENTION

The invention relates to a therapeutic method for treating hypoxia-induced learning and/or memory impairment in a hypoxic subject comprising blocking adenosine A1 receptors in the brain of the subject thereby preventing synaptic arrest of the CA1 neuronal network and maintaining theta activity.

The invention provides a method for relieving hypoxia-induced memory loss in a subject exposed to hypoxia, comprising administering to the brain of the subject an adenosine A1 receptor antagonist in an amount effective to prevent and/or reduce synaptic arrest leading to loss of theta rhythm. The adenosine A1 receptor antagonist can be selected from the group consisting of 8-cyclopentyl-1,3-dipropylxanthine(CPDPX), 1,3-diethyl-8-phenylxanthine (DPX), 8-(p-sulfophenyl)theophylline, BWA-844U, XAC, CGS-15943, BWA-1433U, CP-68,247, XCC, 8-PT, DPSPX and CP-66,713. The hypoxia reduces theta activity by at least about 50% to about 99% and the antagonist mitigates the effects of hypoxia by restoring about 75% to about 100% of pre-hypoxia levels for synaptic transmission and/or theta and intracellular theta activity.

The invention also relates to a therapeutic formulation comprising a pharmaceutically acceptable composition comprising an adenosine A-1 antagonist, the composition delivering the antagonist across the blood brain barrier, the composition not causing any unwanted side effects in concentrations effective to block learning and/or memory-loss related lesions caused by hypoxia. The invention also relates to an article of manufacture consisting essentially of a pharmaceutically acceptable composition, packaged together with instructions indicating use in connection with mitigating hypoxia-induced lesions. The adenosine A1 receptor antagonist can be 8-cyclopentyl-1,3-dipropylxanthine(CPDPX), 1,3-diethyl-8-phenylxanthine (DPX), 8-(p-

sulfophenyl)theophylline, BWA-844U, XAC, CGS-15943, BWA-1433U, CP-68,247, XCC, 8-PT, DPSPX or CP-66,713. The formulation can comprise a combination of adenosine A-1 antagonist with an agent that reverses cellular injury and/or prevents cell loss.

The invention provides a therapeutic method comprising administering to the brain of a subject exposed to hypoxia a pharmaceutical composition comprising an effective amount of an adenosine A1 receptor antagonist, thereby treating or preventing hypoxia-induced learning impairment and/or memory loss and associated synaptic arrest and/or impairment. The associated synaptic arrest can be an impairment of cholinergic theta activity and synaptic transmission in hippocampal CA1, thereby affecting spatial learning and memory.

The invention also relates to a method of treating a neurodegenerative disorder of a subject comprising administering to the subject an effective amount of an adenosine A1 receptor antagonist in combination with an effective amount of an agent that reverses cellular injury and/or prevents cell loss. It can also be a method of maintaining theta activity during hypoxia, comprising administering an adenosine A1 antagonist to brain tissue.

The invention relates to a method of identifying therapeutic A-1 antagonist compounds useful for treating hypoxia-related memory loss comprising: providing brain tissue under controlled conditions modeling theta activity, placing the tissue under conditions of hypoxia causing synaptic arrest and loss of theta activity, administering a candidate A-1 antagonist compound to the brain tissue under conditions of hypoxia, and determining whether the candidate compound prevents synaptic arrest of the brain tissue and/or maintains theta activity. The brain tissue can comprise CA1 pyramidal cells.

Further objectives and advantages will become apparent from a consideration of the description, drawings, and examples.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is better understood by reading the following detailed description with reference to the accompanying figures:

Figures 1a-1, 1a-2, 1a-3, 1b-1, 1b-2, 1b-3, 1c, 1d and 1e show the differential effects of brief hypoxia on cholinergic CA1 θ and long-term potentiation of Sch-CA1 EPSPs. Examples of recorded field potentials: pre-carbachol control (Fig. 1a-1), during carbachol (50uM, 30 min; Fig. 1a-2) and 10 min after brief hypoxia (5% O_2 3min; Fig. 1a-3). Membrane potential traces of recorded CA1 pyramidal cells: pre-carbachol (control; Fig. 1b-1), during carbachol

application (50 μM, 30 min; Fig. 1b-2), and 10 min after brief hypoxia (5% O_2 3min; Fig. 1b-3). The membrane was maintained at pre-carbachol level by passing negative current (the second trace). Representative Sch-CA1 EPSP traces (Fig. 1c) of post-HFS (LTP, 40 min after HFS) and pre-HFS (Control). Representative Sch-CA1 EPSP traces (Fig. 1d) of pre-HFS (Control), post-HFS (LTP, 29 min after HFS) and immediately after brief hypoxia (5% O_2 3min). Fig. 1e represents time course of Sch-CA1 EPSPs in response to HFS (at the first arrow) and brief hypoxia (at the second arrow). Data points are mean±S.E. of the mean. EPSPs were evoked 1/min. For clarity, only every other points are shown ■: control; ●: 5% O_2 for 3 minutes.

Figures 2a, 2b, 2c and 2d demonstrate the synaptic arrest produced by brief hypoxia without causing obvious cellular loss. Sch-CA1 EPSPs (Fig. 2a) and EPSCs (Fig. 2b) were briefly abolished at the end of brief hypoxia (5% O_2 3min), as compared with those of the next trace (Recovery) and of pre-hypoxia (Control). Representative traces of membrane response to local application of glutamate (Glut; 20 μl of 10 mM) before (Fig. 2c) and at the end of brief hypoxia (Fig. 2d); with glutamate application (20 μl of 10 mM) about 0.5 s before the end of the 3 min hypoxia so that the peak was about the end of the 3min). Nissl stained coronal sections of the dorsal CA1 field revealed densely packed pyramidal cells with well-defined nuclei in control rats and rats subjected to 8 episodes of brief hypoxia (not shown).

Figures 3a, 3b, 3c-1, 3c-2, 3c-3, 3d-1, 3d-2 and 3d-3 show the effects of adenosine A_1 receptor antagonist on synaptic arrest, CA1 θ, in response to brief hypoxia. In the presence of citicoline (100 μM), synaptic arrest was observed at the end of the 3 min hypoxia (Fig. 3a). In the presence of 8-cyclopentyl-1,3-dipropylxanthine (10 μM), synaptic arrest was abolished (Fig. 3b) and brief hypoxia neither eliminated cholinergic CA1 θ (Fig. 3c-1-3) nor cholinergic intracellular θ of the CA1 pyramidal cells (Fig. 3d-1-3).

Figures 4a, 4b, 4c, 4d, 4e and 4f demonstrate the effects of brief hypoxia and adenosine A_1 receptor antagonist on rat performance in the hidden platform water maze task. The figure illustrates experimental protocol (Fig. 4a), escape latency (means ± SEM) in water maze training (Fig. 4b) across 12 trials ($F_{11,312}$= 50.14, p<0.0001), and quadrant preference (Fig. 4c, 4d and 4e) conducted at the end of the twelfth training session, and swimming distance (in 1 min; Fig. 4f). Rats were either subjected to air or hypoxia (95% N_2/5% CO_2 for 100 s) about 30 min in a glass jar after the 2nd or 4th trial of the day. Bilateral i.c.v. CPDPX (400 nmoles/site) or vehicle were administered before the 2nd and 4th trials of the day. Quadrant 4 is the target quadrant during training.

DETAILED DESCRIPTION

In describing preferred embodiments of the present invention, specific terminology is employed for the sake of clarity. However, the invention is not intended to be limited to the specific terminology so selected. It is to be understood that each specific element includes all technical equivalents, which operate in a similar manner to accomplish a similar purpose. Each reference cited here is incorporated by reference as if each were individually incorporated by reference.

The invention provides therapeutic methods and compositions targeted to lesions induced by hypoxia leading to memory loss. These lesions have biochemical, physiological, and cognitive aspects, all of which are related and may be considered as targets subject to therapy according to the invention. The biochemical target for the methods and formulations for the invention is the adenosine A-1 receptor in neurons associated with memory and learning, in particular those which are affected by hypoxia. The targeted receptors respond to adenosine signals during hypoxia in a cascade causing synaptic arrest and memory impairment, without cell damage or death.

The physiological aspect of lesions targeted by the invention is the reversible condition of synaptic arrest and reduction of cholinergic theta induction of the CA1 neuronal network. This network includes CA1 pyramidal neurons and others involved in generating stable theta activity and subject to synaptic arrest during hypoxia. During hypoxia, EPSPs and EPSCs are eliminated, the CA1 neuronal network becomes disconnected, and theta activity is reversibly lost until oxygen is applied again.

The cognitive/behavioral lesions subject to therapy according to the invention may be characterized generally as attention impairment, learning impairment, memory impairment, including amnesia, the loss of memory, memory retention, and learning, including spatial learning. The impairment may be sudden as in transient hypoxia, or long term and gradual, or both, as may occur with repeated incidents of transient hypoxia. Such chronic or repeated incidents may lead to other lesions as well.

Subjects in need of the inventive therapy are those exposed to hypoxia from any source. Generally, subjects for therapy are those at risk for hypoxia, including older people, people with chronic obstructive lung disease, people entering surgery, those at risk of stroke, and others having diseases predisposing them to hypoxia. Hypoxia induces many lesions in subjects,

including cell death and a wide variety of synaptic dysfunction. Subjects in need of the therapy are those facing hypoxia-induced theta rhythm abnormality and memory loss.

Only that synaptic dysfunction associated with memory loss is subject to therapy here, and other hypoxia effects are not subject to treatment according to the invention. For example, hypoxia interferes with long term potentiation (LTP) but treatment with an adenosine A1 receptor antagonist does not mitigate that interference. It was not predictable that A1 antagonists would work on CA1 neurons and/or others involved in generating theta activity and supporting memory retention.

The hypoxia subject to therapy according to the invention is mild, i.e. causing reversible effects, but sufficient to interrupt theta activity and/or intracellular theta, without causing cell loss. The hypoxia subject to therapy causes low brain oxygen levels but not substantial immediate cell death. Causes of the hypoxia inducing the lesions targeted by the invention include traumatic events, transient ischemic attack, surgery-related hypoxia, acute and/or chronic obstructive lung disease, central nervous system infections such as meningitis encephalitis and/or other traumatic injury of the central nervous system.

Repeated hypoxic episodes of the type subject to therapy may be associated with and/or precede neurodegeneration over time. Such disorders include Alzheimer's Disease, Parkinson's, Pugilistia or dementia.

The invention reduces or eliminates the lesions induced by hypoxia. The inventive therapy blocks adenosine A1 receptors on the targeted neurons. This blockade protects and enhances synaptic efficacy and eliminates interruption of, or reduces synaptic dysfunction referred to here as synaptic arrest leading to loss of stable theta rhythm. The methods and compositions provide therapy for a condition of impaired memory in a subject exposed to hypoxia, treat or prevent memory loss, blocking or mitigating the extent of the cognitive impairment.

Therapy according to the invention means administering an adenosine A1 receptor antagonist to neurons involved in generating theta rhythm, in an amount effective to prevent synaptic arrest induced by hypoxia. The antagonist must be administered in a dose and manner effective to cross the blood brain barrier to provide a blockade effect at the time it is needed, i.e. during hypoxia.

Another aspect of the invention relates to a method for treating or preventing memory loss by administering an adenosine A1 receptor antagonist, which reduces the effects of

reversible transient hypoxia and associated synaptic dysfunction. The hypoxia effects according to the invention may involve reductions of about 50% to about 95%, e.g. about 75%, about 80%, or about 90%, of synaptic transmission, theta activity and/or intracellular theta activity. Using a selective adenosine A_1 receptor antagonist according to the invention may mitigate the effects of hypoxia by restoring about 75% to about 100%, e.g. about 80, 90, 95 or 99%, of pre-hypoxia levels for synaptic transmission and/or theta and intracellular theta activity.

The formulations of the invention are pharmaceutically acceptable compositions comprising adenosine A-1 antagonists. Particularly useful in the invention are those antagonists which can cross the blood brain barrier and do not cause any unwanted side effects in concentrations effective to block the memory-loss-related lesions caused by hypoxia. According to the invention, a commercial product is provided consisting essentially of such a pharmaceutically acceptable composition packaged together with instructions indicating use in connection with mitigating hypoxia-induced lesions.

The invention provides a method for relieving hypoxia-induced memory loss in a subject exposed to hypoxia, comprising administering to the subject an adenosine A1 receptor antagonist in an amount effective to prevent synaptic arrest leading to loss of theta rhythm. The invention provides a method for preventing hypoxia induced, reversible synaptic arrest in a subject by blocking adenosine A1 receptors in the brain of the subject.

The present invention also relates to a method of treating a neurodegenerative disorder comprising administering an effective amount of an adenosine A1 receptor antagonist (in combination with an effective amount of an agent that reverses cellular injury and prevent cell loss). The invention further relates to a pharmaceutical composition comprising an adenosine A1 receptor antagonist and a pharmaceutically acceptable carrier, the composition delivering the antagonist across the blood brain barrier. In another embodiment, the invention relates to a method of maintaining theta activity during hypoxia, comprising administering an adenosine A1 antagonist to the brain in an effective amount. Another aspect relates to a method of preventing or reversing synaptic arrest of CA1 neurons due to hypoxia in the absence of cellular injury.

Brief hypoxia impairs functioning of CA1 neuronal synaptic transmission, long-term potentiation (LTP) of glutamatergic EPSPs, and cholinergic θ, a memory-related neuronal activity synchronization that depends on a temporal heterosynaptic interaction[5]. In addition, brief hypoxia blocks synaptic transmission[6] of glutamatergic inputs, GABAergic inputs and

cholinergic inputs, causing disconnection, or synaptic 'arrest', of the CA1 neuronal network. Many of these inputs and their interaction play an essential role in enhancing synaptic efficacy in learning and memory.

In experiments conducted by the inventors, brief hypoxia eliminated EPSPs and EPSCs temporarily. The synaptic 'arrest' immediately disappeared when reoxygenation was initiated and was not produced postsynaptically. The hypoxic synaptic 'arrest' and reduction in cholinergic θ induction were prevented by blocking the adenosine A_1 receptors. Application of citicoline, a neuroprotective substance, on the other hand, is ineffective suggesting that cellular injury is not involved.

In the presence of 8-pentyl-1,3-dipropylxanthine (CPDPX), a selective adenosine A_1 receptor antagonist, synaptic transmission remained intact at the end of the hypoxia. Neither the θ activity nor intracellular θ were affected by the brief hypoxia.

The inventors have demonstrated that a selective adenosine A_1 receptor antagonist, such as 8-cyclopentyl-1,3-dipropylxanthine (CPDPX), can be utilized to eliminate the functional impairment associated with transient hypoxia-induced memory loss and associated synaptic dysfunction. This can be achieved by relieving the network from heterosynaptic 'arrest' by blocking the adenosine A_1 receptors.

The present invention further comprises combining the selective adenosine A_1 receptor antagonist with agents that reverse cellular injury and prevent cell loss. In addition, the antagonists might also be valuable in therapy against severe hypoxia/ischemia-induced memory loss.

The present invention is employed to treat disorders of impaired neurotransmission by administering a selective adenosine A1 receptor antagonist in effective amounts. Such disorders may include traumatic brain or spinal cord injury or a neurologic or neuromuscular disease such as myasthenia gravis, multiple sclerosis, Alzheimer's disease, or spinal disorders. In addition, the present invention provides a pharmaceutical composition and a pharmaceutically acceptable carrier.

General methods for blocking adenosine A1 receptors are well known. Many adenosine A-1 antagonists are known and persons having ordinary skill in the art may identify more by conventional screening methods. See U.S. Patent No. 6,166,181 to Jacobson et al., and Joel Linden, *Structure and Function of A1 adenosine receptors*, The FASEB Journal, V5:2668-2676 (September 1991), incorporated herein by reference in their entirety. Particular examples of

adenosine A-1 antagonists are 8-cyclopentyl-1,3-dipropylxanthine(CPDPX), 1,3-diethyl-8-phenylxanthine (DPX), 8-(p-sulfophenyl)theophylline, BWA-844U, XAC, CGS-15943, BWA-1433U, CP-68,247, XCC, 8-PT, DPSPX and CP-66,713.

Therapeutic methods of administering a pharmaceutical composition to the brain of a subject exposed to hypoxia. The chemical compositions useful in the present invention can be "converted" into pharmaceutical compositions by dissolution in, and/or the addition of, appropriate, pharmaceutically acceptable carriers or diluents. Thus, the compositions may be formulated into solid, semi-solid, liquid, or gaseous preparations, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injectables, inhalants, and aerosols, using conventional means. Known methods are used to prevent release or absorption of the active ingredient or agent until it reaches the target cells or organ or to ensure time-release of the agent. A pharmaceutically acceptable form is one that does not inactivate or denature the active agent. In pharmaceutical dosage forms useful herein, the present compositions may be used alone or in appropriate association or combination with other pharmaceutically active compounds.

Accordingly, the pharmaceutical compositions of the present invention can be administered to any of a number of sites of a subject and thereby delivered via any of a number of routes to achieve the desired effect. Local or systemic delivery is accomplished by administering the pharmaceutical composition via injection, infusion or sintillation into a body part or body cavity, or by ingestion, inhalation, or insufflation of an aerosol. Preferred routes of administration include parenteral administration, which includes intramuscular, intracranial, intravenous, intraperitoneal, subcutaneous intradermal or topical routes.

The present compositions can be provided in unit dosage form, wherein each dosage unit, e.g., a teaspoon, a tablet, a fixed volume of injectable solution, or a suppository, contains a predetermined amount of the composition, alone or in appropriate combination with other pharmaceutically active agents. The term "unit dosage form" refers to physically discrete units suitable for a human or animal subject, each unit containing, as stated above, a predetermined quantity of the present pharmaceutical composition or combination in an amount sufficient to produce the desired effect. Any pharmaceutically acceptable diluent or carrier may be used in a dosage unit, e.g., a liquid carrier such as a saline solution, a buffer solution, or other physiologically acceptable aqueous solution), or a vehicle. The specifications for the novel unit dosage forms of the present invention depend on the particular effect to be achieved and the particular pharmacodynamic properties of the pharmaceutical composition in the particular host.

An "effective amount" of a composition is an amount that produces the desired effect in a host, which effect can be monitored, using any end-point known to those skilled in the art. The methods described herein are not intended to be all-inclusive, and further methods known to those skilled in the art may be used in their place.

Brain tissue according to the invention may be in situ (in a subject's brain) or in vitro (e.g. a brain tissue biopsy or slice) under controlled conditions modeling theta activity. Furthermore, the amount of each active agent exemplified herein is intended to provide general guidance of the range of each component which may be utilized by the practitioner upon optimizing these methods for practice either in vitro or in vivo. Moreover, exemplified dose ranges do not preclude use of higher or lower doses as might be warranted in a particular application. For example, the actual dose and schedule may vary depending on (a) whether a composition is administered in combination with other pharmaceutical compositions, or (b) inter-individual differences in pharmacokinetics, drug disposition, and metabolism. Similarly, amounts may vary for in vitro applications. One skilled in the art can easily make any necessary adjustments in accordance with the necessities of the particular situation.

EXAMPLES

Methods

Brain slices and electrophysiology. Male Sprague-Dawley rats (150-200 gm) were anesthetized with pentobarbital and the brains were removed and cooled rapidly in aCSF solution, bubbled continuously with 95% O_2 and 5% CO_2. Hippocampi were sliced (400 μM) and placed in oxygenated aCSF (NaCl, 124 mM; KCl 3; $MgSO_4$ 1.3; $CaCl_2$ 2.4; $NaHCO_3$ 26; NaH_2P0_4 1.25; and glucose 10). The CA1 pyramidal cells were recorded at 30-31 °C with sharp electrodes (tip resistance: 60-120 MΩ). Study was performed on CA1 neurons with stable resting membrane potential more negative than -70 mV. Unless otherwise mentioned, test stimuli were applied at frequency of 1 per minute (0.017 Hz). Signals were amplified with AxoClamo-2B amplifier, digitized and stored using DigiData 1200 with the P-Clamp data collection and analysis software (Axon Instruments, Inc.).

Hypoxia. Episodes of hypoxia were induced by replacing the oxygen supply with 95% N_2 /5% O_2 / 5% CO_2 for 3 min or 95% N_2 /5% CO_2 for 100 s. The neuronal responses to either were found to be identical in preliminary experiments. The hypoxia is milder than those used by others to produce an irreversible impairment of synaptic transmission[25].

Histology. At the end of behavioral testing, the rats were perfused transcardially under deep terminal pentobarbital anesthesia with 400 ml of 10% formaldehyde. Perfused brains were embedded in wax. Coronal 7-μm sections were cut by a rotary microtome and serial sections through the hippocampal formation were mounted on slides, and processed for Nissl staining.

Spatial maze tasks. Male adult Wistar rats (200-250 gm) were anesthetized with sodium pentobarbital (60 mg/kg, i.p) and placed in a stereotactic apparatus (Kopf Instruments, Tujunga, CA). Two stainless steel guide cannulas were placed with the tips positioned at the coordinates (anterior-posterior, 0.5 mm; lateral, 1.5 mm; horizontal, 3.2 mm), under aseptic conditions. A 7-day recovery period was allowed before any further experimentation. All rats were randomly assigned to different groups (10 each) and swam for 2 min in a 1.5 m (diameter) x 0.6 m (depth) pool (22 ± 1 °C). On the following day, rats were trained in a 2 trial per day task for 4 consecutive days. Each training trial lasted for up to 2 min, during which rats learned to escape from water by finding a hidden platform that was placed at a fixed location and submerged about 1 cm below the water surface. The navigation of the rats was tracked by a video-camera. The quadrant test (1 min) was performed after removing the platform, 24 hrs after the last training trial.

Results

Effects of brief hypoxia on functions of CA1 neurons were monitored on synaptic transmission, long-term potentiation (LTP) of glutamatergic EPSPs, and cholinergic θ, a memory-related neuronal activity synchronization that depends on a temporal heterosynaptic interaction. Bath application of carbachol (50μM, 20 min), a cholinergic receptor agonist, to hippocampal slices mimicked septal activation and diffuse acetylcholine transmission and induced CA1 θ field potential (Fig. la; peak amplitude: 0.73±0.02 mV, n=10, $p<0.05$, at 7.4±0.7 Hz from background noise). The θ is sensitive to atropine blockade and lasted for more than 3h[7]. The θ oscillation of membrane potential (7.5±1.0 mV; n=18; $p<0.05$) was also observed in CA1 pyramidal cells (intracellular θ; Fig. lb). Brief hypoxia, induced about 30 min after θ induction, greatly reduced θ activity by 87.4% (±5.2%; n=8, $p<0.05$; Fig. la) and intracellular θ by a similar extent (by 88.2±4.9%, n=9, $p<0.05$; Fig. 1b). LTP of responses to schaffer collateral (Sch) glutamatergic inputs (Fig. 1c,e), however, was not reduced, but enhanced, by the hypoxia (Fig. 1d,e), consistent with reported hypoxic LTP[8] and the observation that LTP expression is not vulnerable to transient hypoxia a few minutes after hypoxia. The synaptic

transmission was briefly blocked only at the very end of the 3 min of hypoxia[9].

The period of hypoxia is known to block synaptic transmission of glutamatergic inputs[8], GABAergic input[8, 10] and cholinergic inputs[11, 12], causing disconnection, or synaptic 'arrest', of CA1 neuronal network[8]. These inputs and their interaction are known to play an essential role in enhancing synaptic efficacy in learning and memory[13]. Effects of brief hypoxia on synaptic transmission and of agents on hypoxic responses were monitored on responses of CA1 pyramidal cells to Sch activation. Brief hypoxia eliminated the EPSPs and EPSCs briefly (Fig. 2a,b; by 95.2±5.6%, n=10, and 96.8±4.2 %, respectively, $p<0.05$)g. The synaptic 'arrest' immediately disappeared when reoxygenation was initiated (Fig. 2a,b) and was not produced postsynaptically. Local application of glutamate during the last few seconds of the 3 min hypoxia revealed a peak inward current (201.2±10.5 pA) that differed insignificantly (n=7, $p>0.05$) from their control value (206.8±9.7 pA).

The hypoxic synaptic 'arrest' and reduction in cholinergic θ induction were prevented by blocking the adenosine A_1 receptors. Application of citicoline, a neuroprotective substance[14], on the other hand, is ineffective (Fig. 3a; n=6,.$p<0.05$), suggesting that cellular injury was not involved. In the presence of 8-cyclopentyl-1,3-dipropylxanthine (CPDPX), a selective adenosine A_1 receptor antagonist, the synaptic transmission remained intact at the end of the hypoxia (Fig. 3b, 99.2±2.4% at the end of hypoxia versus control 100%; n=7,.$p>0.05$). Neither was θ activity (100.2±3.2%, n=6,.$p>0.05$) nor intracellular θ (99.6±3.0%, n=8,.$p>0.05$) affected by the brief hypoxia (Fig. 3c,d).

One of the most persistent consequences of transient hypoxia/ischemia is amnesia. Effects of brief hypoxia and CPDPX on spatial learning (Fig. 4a) were evaluated in rats, using a hidden-platform water maze. The episodes of brief hypoxia did not cause any obvious cell loss (Fig. 3e,f). As shown in Figure 4b, the latency to escape to the platform in all three groups of rats decreased following the training sessions. However, the group difference was significant ($F_{2,27}=9.142$,.$p<0.001$), indicating that spatial learning in rats subjected to brief hypoxia (hypoxia rats) was slower. A *post hoc* analysis reveals a significant difference from the 3rd trials ($p<0.05$). Quadrant tests 24 hrs after the last training trial revealed that the hypoxia rats (Fig. 4d) did not exhibit a quadrant preference ($F_{3,36}=1.8$,.$p>0.05$), whereas the control ($F_{3,36}=160.3$,.$p<0.0001$; Fig. 4c)) spent more time searching in the target quadrant (Quadrant 4) where the platform was previously placed. Thus, hypoxia rats performed worse than their controls in this spatial memory retention task.

The brief hypoxia-induced memory deficits were sensitive to CPDPX. Bilateral intracerebroventricular injections of CPDPX eliminated hypoxic impairment on the spatial memory (Fig. 4b). Quadrant tests revealed that CPDPX-hypoxia rats showed a preference for the target quadrant ($F_{3,36}$=169.7,.p<0.0001; Fig. 4e), identical to that of the control. The total swimming distances, however, did not differ between the three groups (Fig. 4f;.p>0.05).

Factors other than the extent of CA1 cell loss are also known to contribute to behavioral impairments[15, 16]. Transient hypoxia/ischemia induces release of adenosine[17, 19], resulting in opening of both K_{ATP} and K_{Ca}^{2+} channels[20] and decreasing stimulus induced calcium influx into neurons[21] via an action at presynaptic and postsynaptic Al receptors. The reduction in cholinergic θ suggests an impaired temporal interaction of heterosynaptic inputs. For stable θ activity, some level of ongoing activity and interaction of heterosynaptic inputs may be necessary. In addition, adenosine A_1 receptors are linked to G-proteins and perhaps via these facilitate the opening of potassium channels. Internal Ca^{2+} release from an $InsP_3$-sensitive internal store might be involved as a major component of the hypoxic response[22]. CA1 functional interference may underlie the observed spatial memory deficits due to brief hypoxia. The slightly enhanced EPSPs and LTP themselves, on the other hand, are unlikely to cause decreased spatial learning. Spatial learning has been reported to be normal with enhanced CA1 long-term potentiation by twofold in inositol 1,4,5-triphosphate 3-kinase$_A$-deficient mice[23]. The episodes of brief hypoxia may be more relevant to a gradual memory decline during aging or Alzheimer's disease. A brief episode of global ischemia was reported to be sufficient to increase the production of amyloid precursor protein in vulnerable CA1 neurons[24]. The hypoxic 'synaptic arrest' compromises the brain's ability to learn and memorize, which is an unnecessary sacrifice if the hypoxia turns out to be brief. Relieving the network from heterosynaptic 'arrest' through blocking the adenosine A_1 receptors may represent an effective strategy to eliminate the functional impairment. Combined with agents that reverse cellular injury and prevent cell loss, the antagonists might also be valuable in therapy against severe hypoxia/ischemia-induced memory loss.

The embodiments illustrated and discussed in this specification are intended only to teach those skilled in the art the best way known to the inventors to make and use the invention. Nothing in this specification should be considered as limiting the scope of the present invention. The above-described embodiments of the invention may be modified or varied, and

elements added or omitted, without departing from the invention, as appreciated by those skilled in the art in light of the above teachings. It is therefore to be understood that, within the scope of the described features and their equivalents, the invention may be practiced otherwise than as specifically described.

REFERENCES

The following citations are incorporated herein by reference:

1. Grubb, N.R., O'Carroll, R., Cobbe, S.M., Sirel, J. & Fox, K.A. Chronic memory impairment after cardiac arrest outside hospital. *Br. Med. J.* **313,** 143-146 (1996).

2. Semenza, G.L. Perspectives on oxygen sensing. *Cell* **98**, 281-284 (1999).

3. Eu, J.P., Sun, J.H., Xu, L., Stamler, J.S. & Meissner, G. The skeletal muscle calcium release channel: coupled O_2 sensor and NO signaling functions. *Cell* **102,** 499-509 (2000).

4. Nakajima, W., *et al.* Apoptosis has a prolonged role in the neurodegeneration after hypoxic ischemia in the newborn rat. *J. Neurosci.* **20**, 7994-8004 (2000).

5. Sun, M.-K., Zhao, W.Q., Nelson, T.J. & Alkon, D.L. Theta rhythm of hippocampal CA1 neuron activity: gating by GABAergic synaptic depolarization. *J. Neurophysiol* **in press,** 2001.

6. Descarries, L., Gisiger, V. & Steriade, M. Diffuse transmission by acetylcholine in the CNS. *Prog. Neurobiol.* **53**, 603-625 (1997).

7. Huerta, P.T. & Lisman, J.E. Bidirectional synaptic plasticity induced by a single burst during cholinergic theta oscillation in CA1 *in vitro. Neuron* **15**, 1053-1063 (1995).

8. Hammond, C., Crépel, V., Gozlan, H. & Ben-Ari, Y. Anoxic LTD sheds light on the multiple facets of NMDA receptors. *Trends Neurosci.* **17**, 497-503 (1994).

9. Arai, A., Larson, J. & Lynch, G. Anoxia reveals a vulnerable period in the development of long-term potentiation. *Brain Res.* **511**, 353-357 (1990).

10. Congar, P, Khazipov, C.P. & Ben-Ari, Y. Direct demonstration of functional disconnection by anoxia of inhibitory interneurons form excitatory inputs in rat hippocampus. *J. Neurophysiol.* **73,** 421-426 (1995).

11. Kàsa, P., Rakonczay, Z. & Gulya, K. The cholinergic system in Alzheimer's disease. *Prog. Neurobiol.* **52**, 511-535 (1997).

12. Porkka-Heiskanen, T., *et al.* Adenosine: A mediator of the sleep-inducing effects of prolonged wakefulness. *Science* **276**, 1265-1267 (1997).

13. Shulz, D.E., Sosnik, R., Haidarliu, S. & Ahissar, E. A neuronal analogue of state-

dependent learning. *Nature* **403**, 549-553 (2000).

14. Shuaib, A., Yang, Y. & Li, Q. Evaluating the efficacy of citicoline in embolic ischemic stroke in rats: neuroprotective effects when used alone or in combination with urokinase. *Exp. Neurol.* **161**, 733-739 (2000).

15. Gibson, G.E. & Duffy, T.E. Impaired synthesis of acetylcholine by mild hypoxic hypoxia or nitric oxide. *J. Neurochem.* **36**, 28-33 (1981).

16. Jaspers, R.M.A., Block, F., Heim, C. & Sontag, K.-H. Spatial learning is affected by transient occlusion of common carotid arteris (2VO): comparison of behavioral and histopathological changes after '2VO' and 'four-vessel-occlusion' in rats. *Neurosci. Lett.* **117**, 149-153 (1990).

17. Van Wylen, D.G., Park, T.S., Rubio, R. & Berne, R.M. Increases in cerebral interstitial fluid adenosine concentration during hypoxia, local potassium infusion, and ischemia. *J. Cereb. Blood Flow Metab.* **6**, 522-528 (1986).

18. Sun, M.-K. Pharmacology of reticulospinal vasomotor neurons in cardiovascular regulation. *Pharmacol. Rev.* **48**, 465-494 (1996).

19. Sun, M.-K. & Reis, D.J. Hypoxia-activated Ca^{2+} currents in pacemaker neuroes of rat rostral ventrolateral medulla *in vitro*. *J. Physiol. Lond.* **476**, 101-116 (1994).

20. Yamamoto, S., Tanaka, E. & Higashi, H. Mediation by intracellular calcium-dependent signals of hypoxic hyperpolarization in rat hippocampal CA1 neurons *in vitro*. *J. Neurophysiol.* 77, 386-392 (1997).

21. Fredholm, B.B. & Dunwiddie, T.V. How does adenosine inhibit transmitter release? *Trends Pharmacol. Sci.* **9**, 130-133 (1988).

22. Belousov, A.B., Godfraind, J.M. & Krnjevic, K. Internal Ca^{2+} stores involved in anoxic responses of rat hippocampal neurons. *J. Physiol. Lond.* **486**, 547-556 (1995).

23. Jun, K., *et al*. Enhanced hippocampal CA1 LTP but not spatial learning in inositol 1,4,5-triphosphate 3-kinase(A)-deficient mice. *Leanring Memory* **5**, 317-330 (1998).

24. Gervais, F.G., *et al*. Involvement of caspases in proteolytic cleavage of Alzheimer's amyloid-β precursor protein and amyloidogenic Aβ peptide formation. *Cell* **97**, 395-406 (1999).

25. Furling, D., Ghribi, O., Lahsaini, A., Mirault, M.-E. & Massicotte, G. Impairment of synaptic transmission by transient hypoxia in hippocampal slices: improved recovery in glutathione peroxidase transgenic mice. *Proc. Natl. Acad. Sci. USA* **97**, 4351-4356 (2000).

WE CLAIM:

1. A therapeutic method for treating hypoxia-induced learning and/or memory impairment in a hypoxic subject comprising blocking adenosine A1 receptors in the brain of the subject thereby preventing synaptic arrest of the CA1 neuronal network and maintaining theta activity.

2. A method for relieving hypoxia-induced memory loss in a subject exposed to reversible transient hypoxia, comprising administering to the brain of the subject an adenosine A1 receptor antagonist in an amount effective to prevent and/or reduce synaptic arrest leading to loss of theta rhythm.

3. The method of claim 2, wherein the adenosine A1 receptor antagonist is selected from the group consisting of 8-cyclopentyl-1,3-dipropylxanthine(CPDPX), 1,3-diethyl-8-phenylxanthine (DPX), 8-(p-sulfophenyl)theophylline, BWA-844U, XAC, CGS-15943, BWA-1433U, CP-68,247, XCC, 8-PT, DPSPX and CP-66,713.

4. The method of claim 2, wherein the hypoxia reduces theta activity by at least about 50% to about 99%.

5. The method of claim 2, wherein the antagonist mitigates the effects of hypoxia by restoring at least about 75% to about 100% of pre-hypoxia levels for synaptic transmission and/or theta and intracellular theta activity.

6. A therapeutic formulation comprising a pharmaceutically acceptable composition comprising an adenosine A-1 antagonist, the composition delivering the antagonist across the blood brain barrier, the composition not causing any unwanted side effects in concentrations effective to block learning and/or memory-loss related lesions caused by hypoxia.

7. An article of manufacture consisting essentially of a pharmaceutically acceptable composition according to claim 6, packaged together with instructions indicating use in connection with mitigating hypoxia-induced lesions.

8. The formulation of claim 6, wherein the adenosine A1 receptor antagonist is selected from the group consisting of 8-cyclopentyl-1,3-dipropylxanthine(CPDPX), 1,3-diethyl-8-phenylxanthine (DPX), 8-(p-sulfophenyl)theophylline, BWA-844U, XAC, CGS-15943, BWA-1433U, CP-68,247, XCC, 8-PT, DPSPX and CP-66,713.

9. The formulation of claim 6, further comprising in combination with the adenosine A-1 antagonist, an agent that reverses cellular injury and/or prevents cell loss.

10. A therapeutic method comprising administering to the brain of a subject exposed to reversible transient hypoxia a pharmaceutical composition comprising an effective amount of an adenosine A1 receptor antagonist, thereby treating or preventing hypoxia-induced learning impairment and/or memory loss and associated synaptic arrest and/or impairment.

11. A therapeutic method according to claim 10, wherein the associated synaptic arrest is an impairment of cholinergic theta activity and synaptic transmission in hippocampal CA1, thereby affecting spatial learning and memory.

12. A method of treating a neurodegenerative disorder of a subject comprising administering to the subject an effective amount of an adenosine A1 receptor antagonist in combination with an effective amount of an agent that reverses cellular injury and/or prevents cell loss.

13. A method of maintaining theta activity during hypoxia, comprising administering an adenosine A1 antagonist to brain tissue.

14. A method of identifying therapeutic A-1 antagonist compounds useful for treating hypoxia-related memory loss comprising:

providing brain tissue under controlled conditions modeling theta activity,

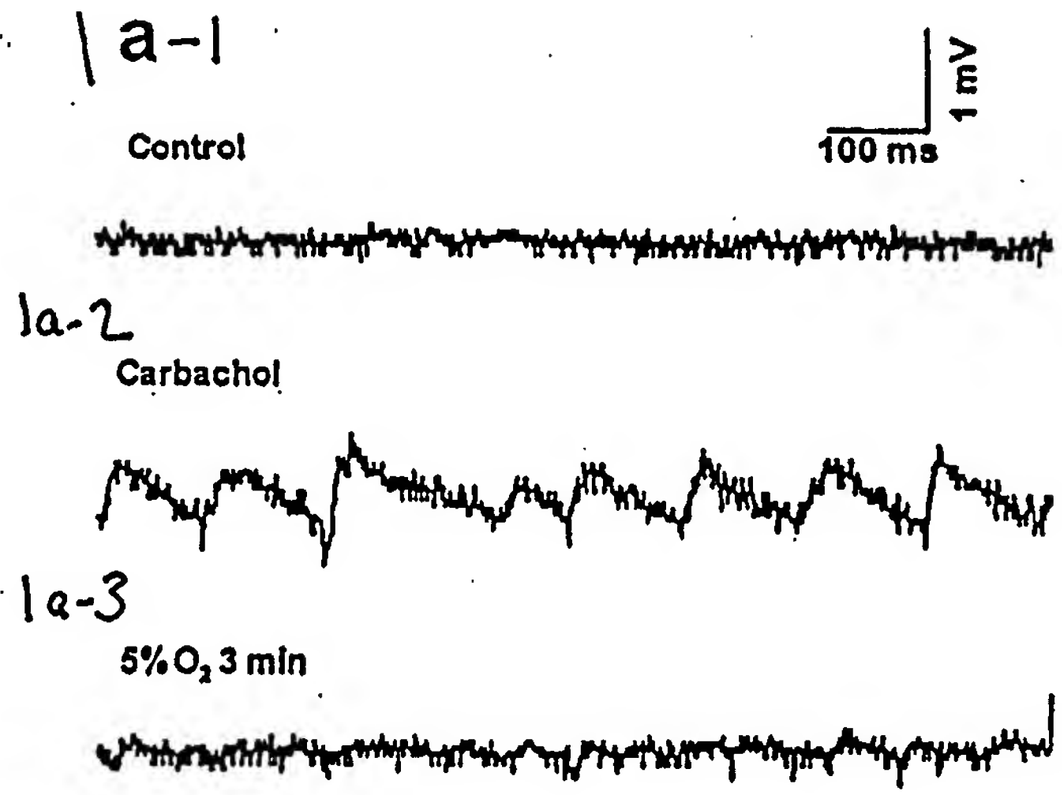
placing the tissue under conditions of hypoxia causing synaptic arrest and loss of theta activity,

administering a candidate A-1 antagonist compound to the brain tissue under conditions

of hypoxia, and

determining whether the candidate compound prevents synaptic arrest of the brain tissue and/or maintains theta activity.

15. The method of claim 14, wherein the brain tissue comprises CA1 pyramidal cells.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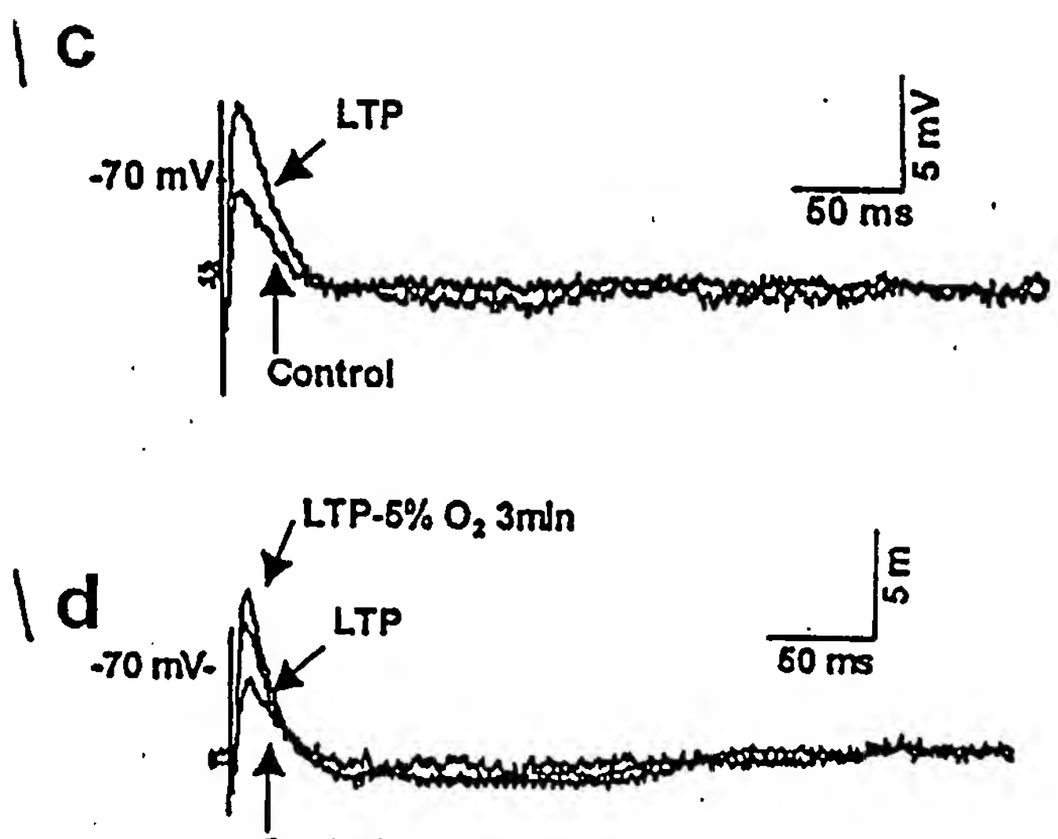
1 mV
a-2
Carbachol
a-3
5% O_2 3 min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50 ms
5 mV
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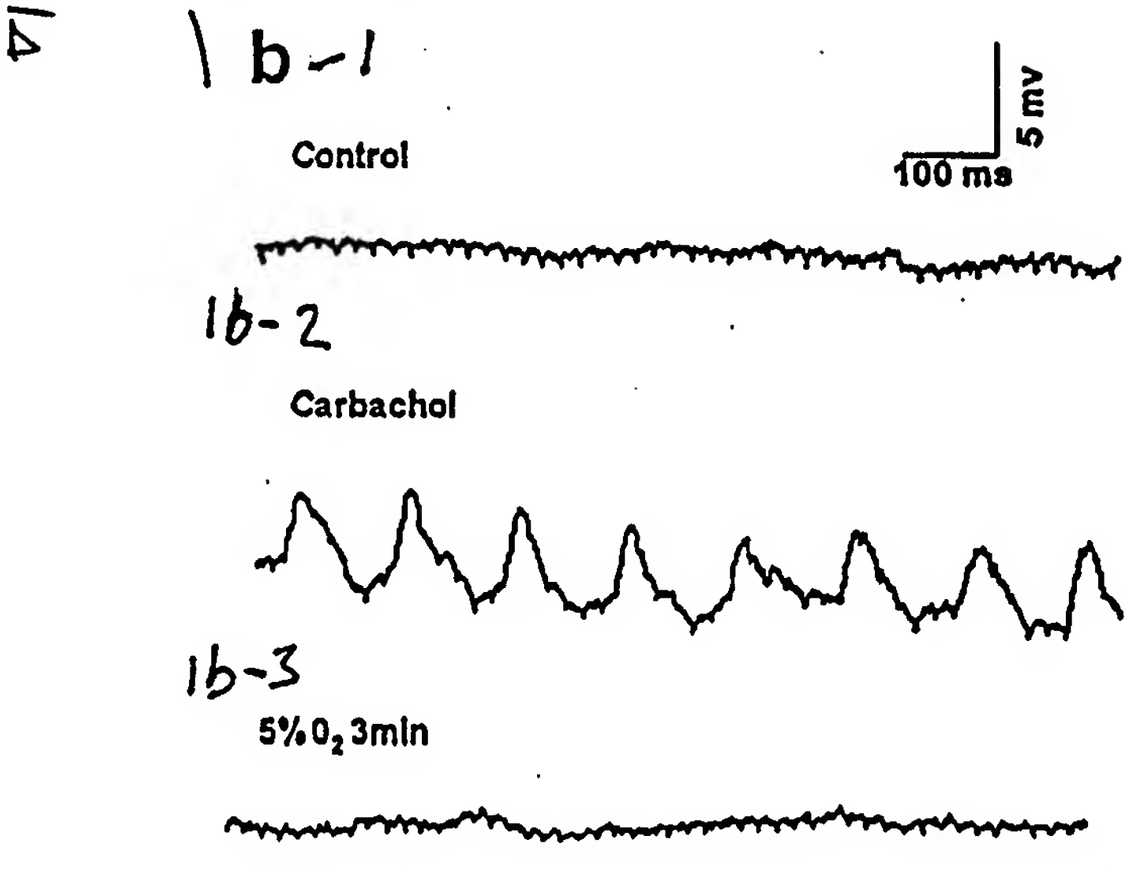
d
LTP-5% O_2 3min
LTP
-70 mV-
50 ms
5 m
Control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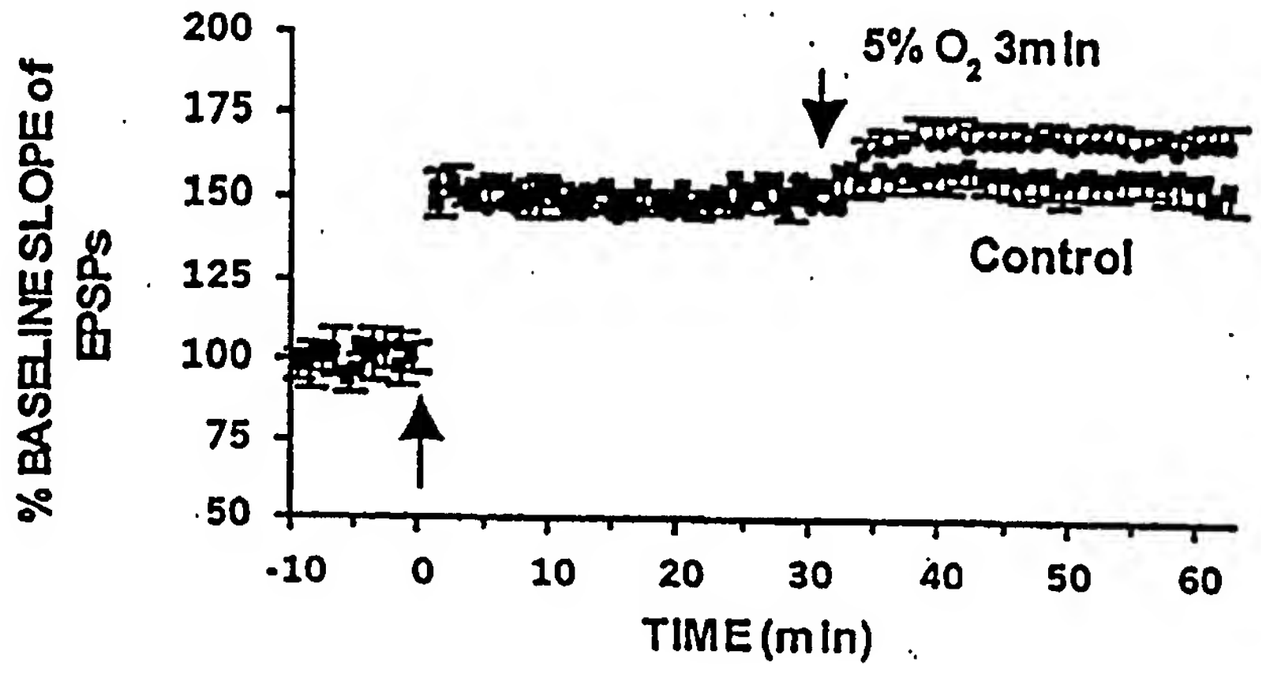
b-3
5% O_2 3min


e
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175
150
125
100
75
50
-10
0
10
20
30
40
50
60
TIME (min)
5% O_2 3min
Control

2c
Glut
200 pA
300 ms
d
Glut
Vh = -75 mV
2a
Recovery
Control
-70 mV
6% O2 3min
5 mV
50 ms
2b
6% O2 3 min
Vh = -76 mV
Control
Recovery
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50 ms

Figure2a-2D

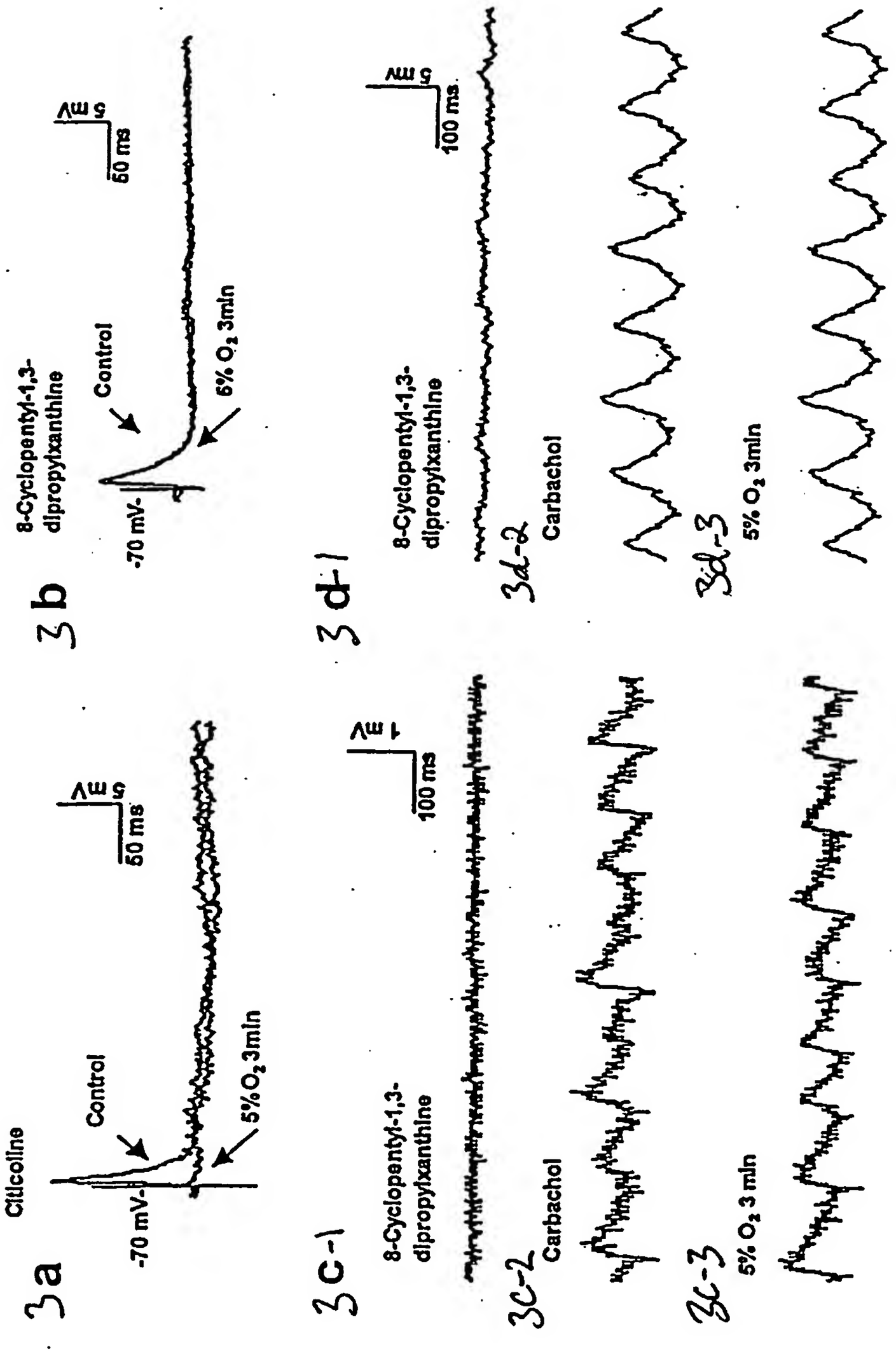
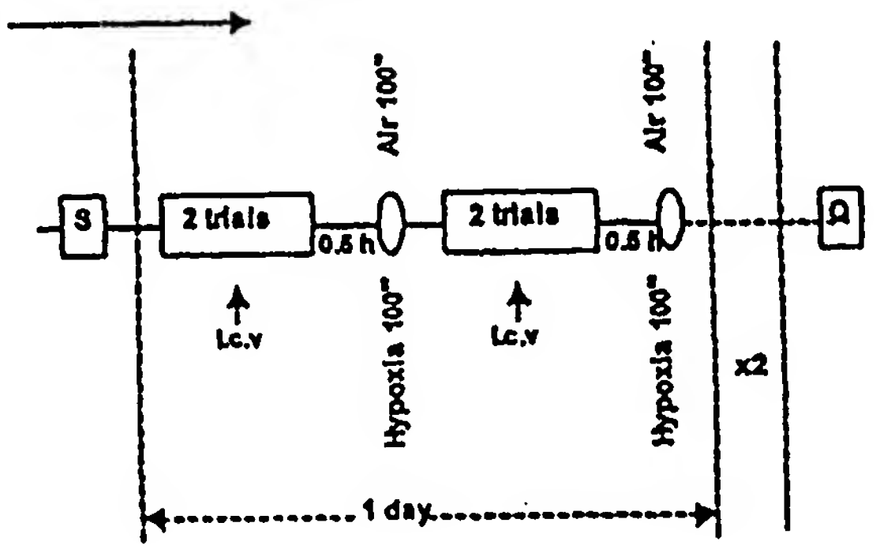


Figure 3a - 3d-3

4 a



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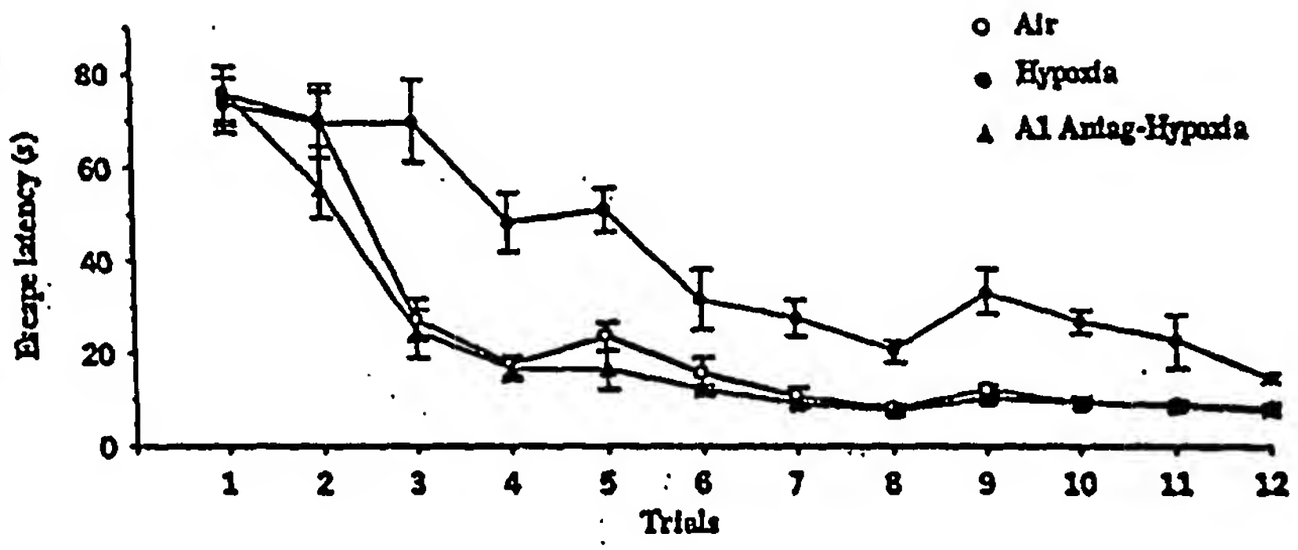

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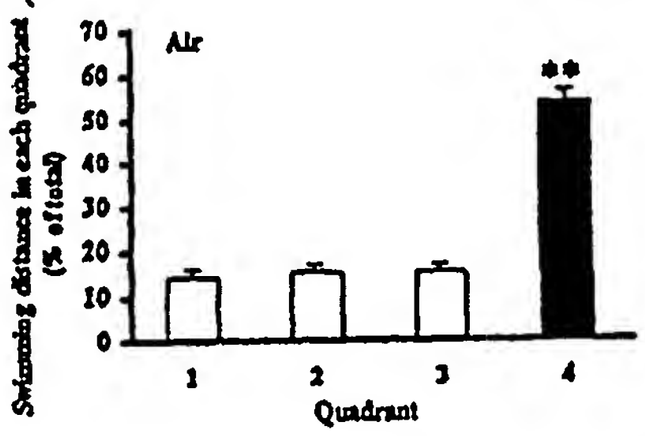

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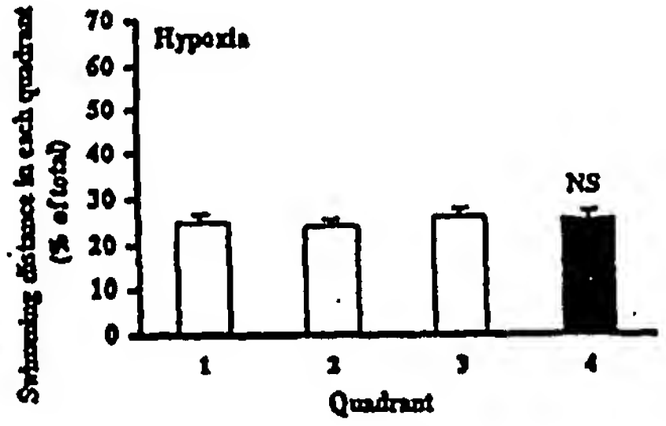

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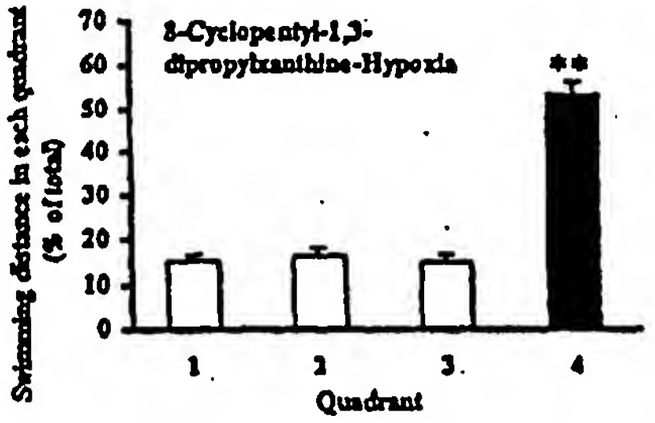

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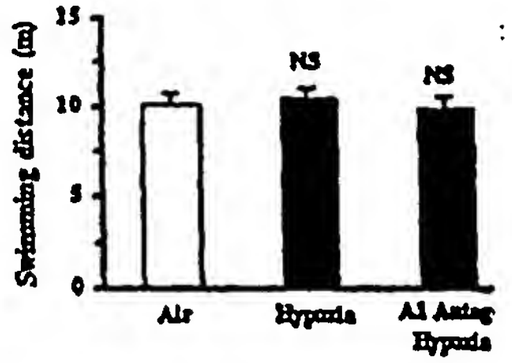

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Figures 4 a - f

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(71) Applicant *(for all designated States except US)*: **BLANCHETTE ROCKEFELLER NEUROSCIENCES INSTITUTE** [US/US]; 9601 Medical Center Drive, Rockville, MD 20850 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **SUN, Miao-Kun** [US/US]; 16012 Howard Landing Drive, Bethesda, MD 20878 (US). **ALKON, Daniel, L.** [US/US]; 6701 Bonaventure Court, Bethesda, MD 20817 (US).

(74) Agent: GOLLIN, Michael, A.; Venable, Baetjer, Howard & Civiletti, LLP, 1201 New York Avenue, NW, Suite 1000, P.O. Box 34385, Washington, DC 20043-9998 (US).

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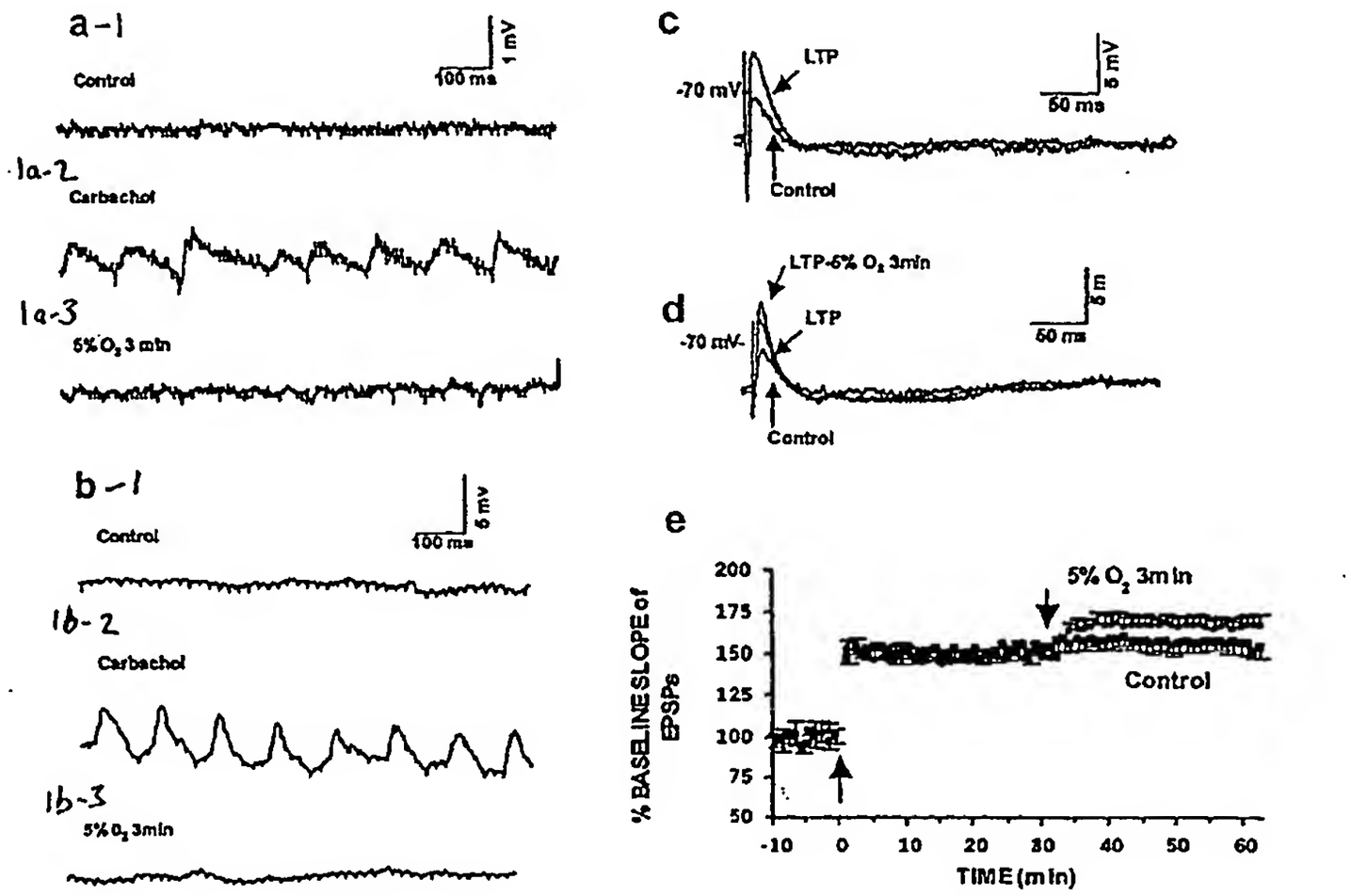
[Continued on next page]

(54) Title: ADENOSINE A1 RECEPTOR ANTAGONISTS FOR TREATING HYPOXIA-INDUCED LEARNING AND MEMORY IMPAIRMENT



(57) Abstract: The invention provides therapeutic methods and compositions comprising adenosine A1 antagonists for treating memory loss produced by reversible transient hypoxia and associated synaptic dysfunction.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US02/14378

A. CLASSIFICATION OF SUBJECT MATTER

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US CL : 514/260, 263

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 514/260, 263

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS on-line

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	TABATA et al., "Ameliorative Effects of Paeoniflorin, a Major Constituent of Peony Root, on Adenosine A1 Receptor-Mediated Impairment of Passive Avoidance Performance and Long Term Potentiation in the Hippocampus", Biol. Pharm. Bull., Vol.24, No. 5, 496-500 (2001), see entire document.	6, 8 1-5, 7, 10, 11
Y	Database Biosis, Abstract number PREV199900424583, Ochiishi et al., "High level of adenosine A1 receptor-like immunoreactivity in the CA2/CA3a region of the adult rat hippocampus", Neuroscience, 93(3), 955-967, 04 August 1999.	13-15
X Y	US 6,187,780 B1 (BLECH et al.) 13 February 2001, see entire document.	6-9 1-5, 10-12

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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